

# Quantitation of Soy Protein in Frankfurters by Gel Electrophoresis

## ABSTRACT

A modified Laemmli procedure was utilized to obtain gel electrophoretic resolution of protein components in soy and beef isolates and in processed meat products containing soy protein isolate (SPI). Height and area ratios of selected soy and beef components ( $\alpha'$ ,  $\alpha$ -subunits of  $\beta$ -conglycinin and actin) were calculated from densitometric scans and compared with similar ratios calculated for soy and beef mixtures of known composition. This method permitted the quantitation of soy protein ( $\pm 0.14\%$ ) in raw and pasteurized frankfurters and should be applicable to other selected soy-containing meat products.

## INTRODUCTION

THE ADDITION of soy protein to a variety of processed meat products has been a practice for many years. While soy products can be legally incorporated into many foods, their use in selected meat products is either disallowed or restricted to specified limits and regulated by product standards and specifications. Numerous methods have been developed for the quantitation of soy protein in processed products based on electrophoretic, immunochemical and nonprotein component analysis (Olsmann and Hitchcock, 1980). Nevertheless, at the present time, no one method fully meets the requirements of regulatory agencies or food processors.

Many electrophoretic methods have been reported for quantifying soy protein in meat products (Hofmann and Penny, 1971; Hofmann, 1973; Guy et al., 1973; Lee et al., 1975, 1976; Armstrong et al., 1982; Parsons and Laurie, 1972; Molander, 1982). A recent international collaborative study (Olsmann et al., 1985) utilizing a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method and an enzyme-linked immunoadsorption assay (ELISA) for the quantitative analysis of soya protein in meat products indicated that significant progress in methodology development had occurred but further refinements were necessary to make them fully acceptable for general product surveillance.

In this study, the results of the application of a Laemmli SDS-PAGE (Laemmli, 1970) procedure as modified by Basch et al. (1985) to quantitate soy in a variety of commercial and prepared products containing known amounts of soy protein isolate (SPI) are reported.

## MATERIALS & METHODS

### Sample preparation

Beef protein isolates were prepared from whole round of beef after dissecting collagenous material from muscle followed by grinding it in a meat grinder and lyophilization. The freeze-dried sample was then extracted three times with 5 volumes of acetone and air-dried at room temperature. Final traces of acetone were removed under vacuum. The defatted samples were stored at refrigeration temperature ( $4^{\circ}\text{C}$ ).

A basic beef formulation was used to prepare test frankfurters (Whiting et al., 1984). Soy-beef frankfurters were prepared by blending ingredients of a standard all beef formulation with commercial low-fat soy isolate to give final soy concentrations of 0–5%. A commercial soy isolate (Supro 620, Ralston Purina Co., St. Louis, MO) was used for

preparation of frankfurters containing soy and as a reference SPI. The frankfurters were smoked by an atomized liquid smoke, cooked in a humidity controlled smokehouse to an internal temperature of  $71^{\circ}\text{C}$ , and cooled with a water spray. Portions of each test batch were retained as uncooked frankfurters for comparative evaluation. Protein, fat and water contents of the frankfurters were determined by Kjeldahl, Soxhlet and oven-drying procedures, respectively (AOAC, 1975).

### Protein determination

The protein content of various beef, soy, and frankfurter sample solutions dispersed in 0.05M TRIS-HCl, pH 8.0, containing 8 M urea, 3.0% SDS, 2.0% 2-mercaptoethanol (solubilizing buffer) was determined by biuret analysis (Lee et al., 1975; Layne, 1957). It was necessary to separate the protein from the components of the solubilizing buffer before proceeding with the biuret determination because of the interference of the buffer components in the biuret procedure. This was done by precipitating the protein contained in a 3.0 mL aliquot with 15 mL of acetone. The precipitated protein was separated by centrifugation and washed three times with additional acetone and air-dried under a stream of nitrogen. Three and one half milliliters of 0.5N NaOH were added to the protein in each tube, mixed well and placed in a boiling water bath for 10 min. After cooling, samples were transferred to a 5-mL volumetric flask and brought to volume with 0.5N NaOH. Protein was calculated as the average value of three different aliquots using bovine serum albumin as the standard. Dried and defatted protein samples were solubilized by adding 100 mg of the sample to about 8 mL 0.5N NaOH and treated as above.

### Gel electrophoresis

Samples removed from the inner core of raw or cooked frankfurters (ca. 20g) were homogenized in 200 mL acetone in a Sorvall Omni mixer for 1 min, then centrifuged at  $18,000 \times g$  for 15 min. The procedure was repeated twice and the pellet allowed to air dry. Approximately 200 mg acetone extracted protein was suspended in 30 mL of solubilizing buffer and blended in a Polytron homogenizer for 1 min and then shaken for 2 hr. The solutions were then centrifuged for 20 min at  $35,000 \times g$  at  $20^{\circ}\text{C}$  and filtered through Whatman No. 4 filter paper. Solubilizing buffer containing 0.1% bromphenol blue was then added before applying protein samples to the gel. Electrophoresis was performed according to the method of Laemmli (1970) as modified by Basch et al. (1985) using either a Hoefer or E-C Vertical Slab Gel apparatus. The whole gel consisted of two portions: a stacking gel (3% acrylamide, 0.08% bis-acrylamide) and a running gel (10% acrylamide, 0.26% bis-acrylamide). Following electrophoresis, gels were stained for protein with 0.03% Coomassie Blue in 10% trichloroacetic acid/10% methanol/7% acetic acid for 16 hr for the E-C gel and for 1 hr for the Hoefer gel. Gels were destained with 10% methanol/7% acetic acid. Measurement of peak heights and peak areas of separated proteins was performed using a Shimadzu Dual-Wavelength Scanner, Model CS-930.

The relative percent actin to the total protein was determined from area measurements by densitometric scanning of patterns of 5 all-beef frankfurters, free of non-meat proteins (labeled ingredients) and lean beef preparations (Anderson, 1981).

## RESULTS & DISCUSSION

APPLICATION of the modified Laemmli SDS-PAGE slab gel procedure results in an enhanced degree of electrophoretic resolution between beef and soy protein components (Fig. 1) that permits quantitative analysis by densitometric scanning. The major electrophoretic components selected for quantitation were beef actin ( $M_r = 43,000$ ) and the  $\alpha'$ - and  $\alpha$ -subunits of soy

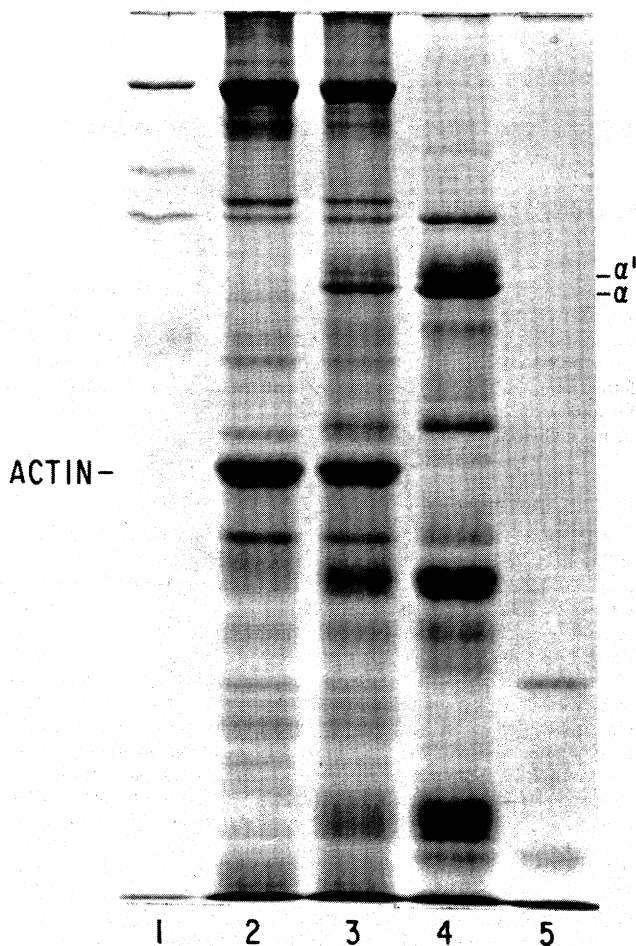


Fig. 1—SDS-PAGE patterns (Hoefer) of (1) molecular weight standards from top to bottom: myosin,  $M_r = 200,000$ ;  $\beta$ -galactosidase,  $M_r = 116,250$ , and phosphorylase B,  $M_r = 92,500$ ; (2) beef protein isolate; (3) frankfurter containing 5% SPI; (4) SPI; and (5) molecular weight standards carbonic anhydrase, (top)  $M_r = 31,000$  and soybean trypsin inhibitor, (bottom)  $M_r = 21,500$ . Actin (A) and the  $\alpha'$ -,  $\alpha$ - $\beta$ -conglycinin components ( $\alpha'$ ,  $\alpha$ ) were used for quantitation of soy-beef protein ratios. Eighty  $\mu$ g protein were applied to each lane.

$\beta$ -conglycinin (CG) (Coates et al., 1985; Thanh and Shibasaki, 1977), ( $M_r = 67,700$  and  $64,600$ , respectively). The  $\alpha$ -CG subunit was used by Armstrong et al. (1982) as a basis for densitometric quantitation of soy protein in conjunction with an internal haemocyanin standard. Actin and a low molecular weight pair of soy components ( $M_r = \text{ca. } 20,000$ ) were used for quantitation in an electrophoretic method reported by Lee et al. (1975). In the present study, ratios of soy and beef protein concentration were determined by: (1) ratios of the peak heights of the  $\alpha$ -subunit and actin; (2) the ratio of the sum of  $\alpha'$ - and  $\alpha$ -subunit peak areas relative to the actin peak area; and (3) ratio of the peak area of the  $\alpha$ -subunit relative to that of actin. These ratios were determined from densitometer scans of gels run on soy protein isolate, beef protein isolate and their mixtures (Fig. 2).

In a separate series of analyses (data not shown), actin relative to the total beef protein ranged from 19.5–22.8% in commercial frankfurters and from 21.5–22.8% in lean beef. The data demonstrated that the actin peak area could be used as a good measure of beef protein.

The superimposed densitometer scans of soy and beef protein isolates (Fig. 3) demonstrate the clear separation and absence of overlapping components in the areas of the  $\alpha$ -,  $\alpha'$ -CG and actin. A plot of ratios of peak heights of  $\alpha$ -CG and actin vs the percent concentration of soy in mixtures of soy

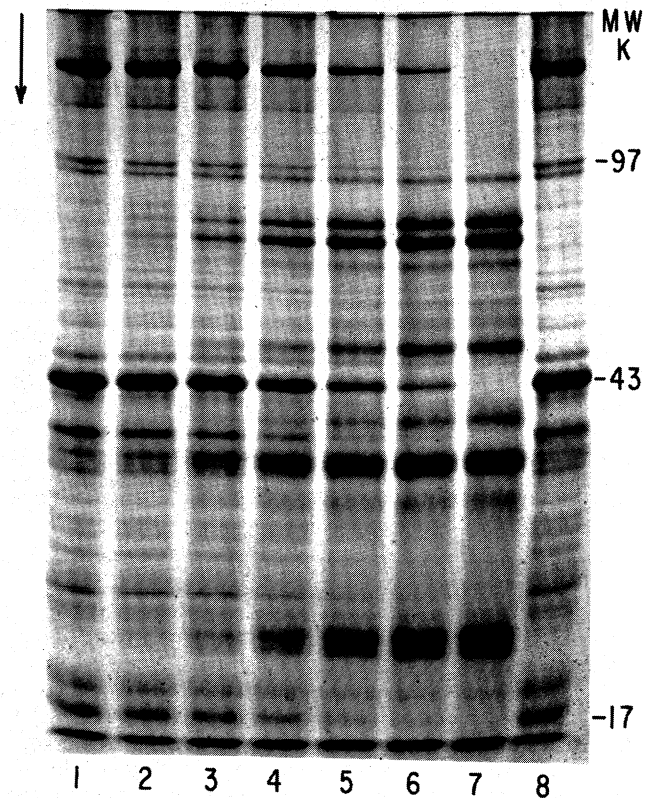


Fig. 2—SDS-PAGE patterns (EC) of beef and soy protein blends. (1) beef isolate; (2–6) beef isolate-SPI blends containing 10, 25, 50, 75, and 90% SPI, respectively; (7) SPI; (8) molecular weight standards.

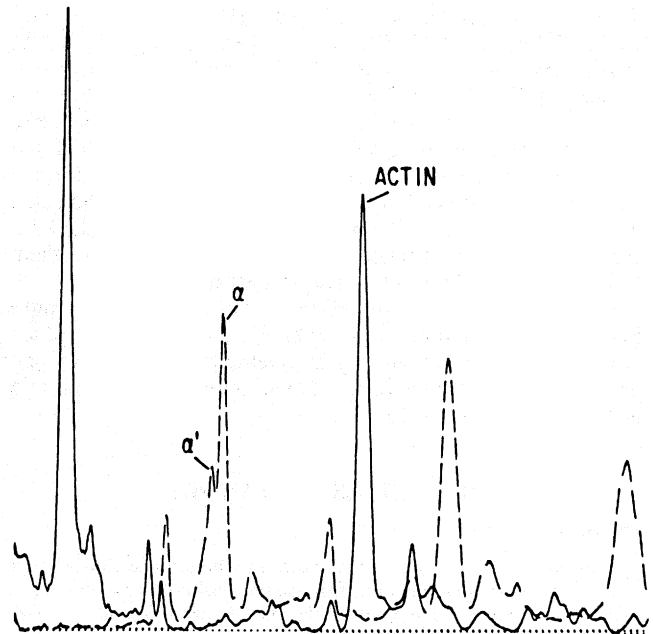


Fig. 3—Densitometric scans of gel patterns of beef isolate (—) and SPI (---). Peaks used for height and area measurements are labelled.

and beef protein isolates (Fig. 4) demonstrate good correlation between these ratios. Such calculations can be used, therefore, to determine soy protein. A plot of the ratios of area of  $\alpha$ -CG alone and  $\alpha' + \alpha$ -CG vs actin demonstrated a similar correlation (data not shown). The results of calculating soy protein in frankfurters containing from 1–5% SPI, from the protein

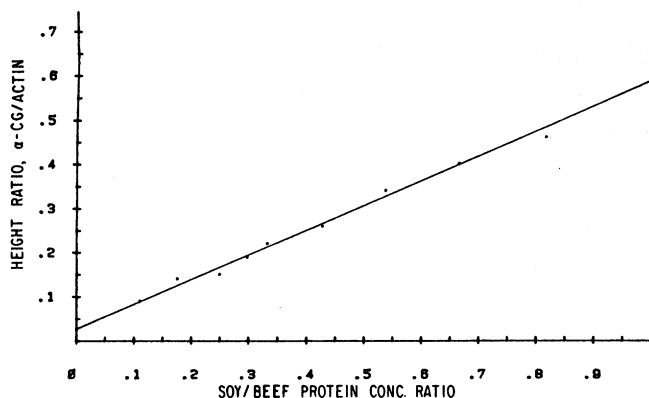


Fig. 4—Plot ratio of peak heights vs percentage soy protein concentration. Correlation coefficient 0.978.

Table 1—Determination of soy isolate<sup>a</sup> in frankfurters

% Soy added	Calculated Soy Protein Content		
	Ratio Used		
	Height $\alpha$ -CG/Actin	Area $\alpha'$ + $\alpha$ -CG/Actin	Area $\alpha$ -CG/Actin
1	1.23 $\pm$ 0.07 (2) <sup>b</sup>	—	—
2	2.15 $\pm$ 0.25 (6)	2.21 $\pm$ 0.005 (5)	2.41 $\pm$ 0.18 (3)
3	3.02 $\pm$ 0.18 (7)	3.36 $\pm$ 0.16 (5)	3.27 $\pm$ 0.12 (3)
4	3.76 $\pm$ 0.11 (7)	4.16 $\pm$ 0.16 (5)	4.18 $\pm$ 0.17 (3)
5	4.75 $\pm$ 0.18 (6)	5.58 $\pm$ 0.18 (5)	5.77 $\pm$ 0.04 (3)

<sup>a</sup> Calculated as percent product weight.

<sup>b</sup> Numbers in parenthesis indicate number of identical samples analyzed in replicate scans.

concentration ratios, is presented in Table 1. Best results were obtained for the full range of samples analyzed by utilizing the  $\alpha$ -CG/actin peak height ratios. The two alternate approaches, while providing reasonable quantitation in the 2–4% range, did not provide sufficient sensitivity for products with 1% or less soy protein and values for the 5% soy product ranged from 10–15% above actual soy protein. No differences in results were observed between raw frankfurters and those cooked to an internal temperature of 71°C (data not shown). The simplicity of calculating ratios of peak heights makes this approach more desirable than using peak areas where variables such as amount of protein applied to the gel, degree of resolution and band curvature can all affect absorbance readings by the densitometer.

Overall, application of the modified Laemmli slab gel electrophoretic method provides a degree of resolution above that obtained without the stacking gel. In terms of ease of calculation and quality of results obtained, the ratio of peak heights

of  $\alpha$ -CG and actin offers the best approach to soy protein quantitation in processed meat products. Similarly, the development of thin gel, multi-lane techniques offers an efficiency of time per sample analyzed comparable to or better than immunochromatographic techniques.

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Ms received 3/6/87; revised 7/1/87; accepted 7/22/87.

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